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Potassium Channel Activation: A Potential Therapeutic Approach?

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ABSTRACT. The physiological role of K^+ channel opening by endogenous substances (e.g., neurotransmitters and hormones) is a recognised inhibitory mechanism. Thus, the identification of novel synthetic molecules that 'directly' open K^+ channels has led to a new direction in the pharmacology of ion channels. The existence of many different subtypes of K^+ channels has been an impetus in the search for new molecules demonstrating channel and, thus, tissue selectivity. This review focuses on the different classes of openers of K^+ channels, the intracellular mechanisms involved in the execution of their effects, and potential therapeutic targets. PHARMACOL. THER. 70(1): 39–63, 1996.

KEY WORDS. Potassium channel openers, KCO classification, K_{ATP}, K_{Ca}, cromakalim, therapeutic targets.

CONTENTS

ABBREVIATIONS. BK_{Ca}, high-conductance calcium-activated potassium channel; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; cAMP, cyclic AMP; cGMP, cyclic GMP; CGRP, calcitonin gene-related peptide; DHS-I, dehydrosoyasaponin I; DMPP, 1,1-dimethyl-4-phenylpiperazinium; EDHF, endothelium-derived hyperpolarizing factor; EDRF, endothelium-derived relaxing factor; E_K, potassium equilibrium potential; ET-1, endothelin-1; 5-HT, 5-hydroxytryptamine; K_{ATP}, ATP-sensitive potassium channel; K_{Ca}, calcium-activated potassium channel; $K_{Ca}CO$, calcium-activated potassium channel opener; KCO, potassium channel opener; $K_{ATP}CO$, ATP-sensitive potassium channel opener; NANCe, nonadrenergic, noncholinergic excitatory neurones; NDP, nucleotide diphosphate; NO, nitric oxide; PGI_2, prostacyclin; pS, conductance; SK_{Ca}, small-conductance calcium-activated potassium channel; SMC, smooth muscle cells; VOC, voltage-operated Ca^{2+} channel.

1. INTRODUCTION

The biological cell is an integral structure that responds to chemical and physical extracellular signals on its membrane, which are communicated to the intracellular processes through a variety of pathways. The transmembrane movement (i.e., efflux and influx) of ions (e.g., Ca^{2+}, Na^+, K^+, Cl^-) through plasmalemma channels are universal mechanisms used to execute or modulate physiological functions in living cells.

Potassium (K^+) specific channels are a diverse and ubiquitous group of ion channels and, thus, play a fundamental role in the modulation of cell excitability (Hille, 1984; Rudy, 1988). In the resting state of excitable or nonexcitable cells, the concentration of K^+ outside the membrane (3–5 mM) is at least 25-fold lower than the K^+ concentration in the intracellular fluid (130–160 mM). Consequently, an outward current due to efflux of positively charged ions is generated by the opening of K^+ channels. The efflux of K^+ is a mechanism for recovering (repolarization), maintaining (clamping), and/or enhancing (hyperpolarization) the resting potential of the cell. Thus, the opening of K^+ channels is a physiological means for counteracting, restricting, or preventing depolarizing activity caused by inward currents, due to entry of Ca^{2+} and Na^+ and the efflux of Cl^- ions.

The functions of these channels, which are crucial for subserving different physiological functions, depend on the

specific manner in which a particular K^+ channel opens and closes, and its selective permeation by K^+ ions. K^+ channels are generally classified according to their primary regulatory or gating mechanism (Hille, 1984). Ion channels can be characterised by ionic selectivity (differential permeability), conductances (pS), gating properties (factors controlling channel opening and closing), kinetics (rates at which channels open and close), and pharmacology (action of specific agents in blocking or changing the flow of ions). K^+ channel classifications and their pharmacological properties have been reviewed extensively (Rudy, 1988; Castle et al., 1989; Cook, 1990; Dreyer, 1990; Kolb, 1990; Robertson and Steinberg, 1990; Aronson, 1992). This article, however, will focus on the subtypes of K^+ channels (e.g., ATP-sensitive potassium channel [K_{ATP}], calcium-activated potassium channel [K_{Ca}]) that have been associated with synthetic and endogenous openers of those channels.

The discovery and development of selective ligands that interact with specific K^+ channels, together with the combination of recent electrophysiological and molecular biology techniques, has resulted in a more detailed characterization of the role these channels play in regulating cell function. An appreciation of the primary amino acid sequence of each K^+ channel protein, through molecular biology techniques, will allow the development of highly selective ligands that open a designated channel subtype. Such ligands would provide essential information regarding the physiological and pathophysiological importance of particular K^+ channels; thus, leading to the identification of drugs focused for defined clinical conditions.

The main objective of this review is to present the different classes of agents that open K^+ channels and discuss potential clinical targets.

2. POTASSIUM CHANNEL OPENER CLASSIFICATION

The emergence of synthetic potassium channel openers (KCOs) has only occurred recently; however, the physiological role of K^+ channel opening by endogenous substances (neurotransmitters and hormones) is a recognized inhibitory mechanism (Kurachi et al., 1986; Bulbring and Tomita, 1987). The term "potassium channel openers" was introduced to describe a group of novel synthetic molecules, typified by cromakalim (Hamilton et al., 1986; Hamilton and Weston, 1989), that have led to a new direction in the pharmacology of ion channels. Hamilton et al. (1986), reporting that cromakalim evoked smooth muscle relaxant effects by the opening of K^+ channels in cell membranes, initiated major research efforts in the search for other such molecules and in the determination of the specific channel(s) involved. KCO properties have, subsequently, been demonstrated in a diverse range of synthetic chemical structures and endogenous substances. In addition, the existence of so many different subtypes of K^+ channel has been an impetus to the search of new molecules that would have profiles and channel selectivities different from those exhibited by the group of KCOs typified by cromakalim. Recent progress has been reported in the search for agents that 'selectively' open calcium-activated K^+ channels (see Section 2.2). In addition to the development of synthetic molecules, a number of endogenous substances have been identified that exert some or all of their effects via K^+ channel opening (see Section 2.3).

The recent advances made in this area of research have brought into question the use of the broad term "potassium channel openers" for reference to a limited group of molecules (i.e., K_{ATP} openers, those typified by cromakalim). For the purpose of this review, the term "potassium channel opener" (and the abbreviation KCO) will be used in reference to any substance reported to open K^+ channels and, thus, associated with an efflux of K^+ ions from the cell. A subscript to the K of the abbreviation KCO will be used to indicate the postulated channel involved (e.g., K_{ATP}CO for openers of K_{ATP} channels).

Molecules (primarily K_{ATP}COs) exhibiting properties compatible with the opening of K^+ channels have been essential tools in the development of research approaches and pharmacological techniques within this area. Such techniques not only have assisted in the identification of other KCOs, but are crucial in the determination of the mechanism(s) of action and K^+ channel subtype involved.

The unidirectional movements of specific ions can be assessed by isotope flux techniques. The efflux of K^+ from a cell can be measured by using $^{42}K^+$ or the more stable tracer $^{86}Rb^+$. Briefly, the tissue or cells are loaded with the isotope during an incubation period (Quast and Baumlin, 1988). The preparation is then challenged with a KCO and the radioactivity within the bathing environment and the residual radioactivity in the tissue is determined, thereby giving qualitative and quantitative representation of K^+ ion movements. In experimental models where mechanical responses can be recorded, the K^+ flux can be compared with functional responses following exposure of the tissue to the KCO.

KCOs hyperpolarise the cell membrane up to values approaching the potassium equilibrium potential (E_K; see Section 3.1). Electrophysiological techniques permit the recording of transmembrane ion movements and membrane potential, depending on the experimental methodology, at a high level of resolution. Patch-clamp procedure applied to whole-cell confirmation allows macroscopic K^+ currents to be recorded when depolarizing pulses are delivered to the preparation clamped at negative potentials close to E_K (Hamill et al., 1981). Recording from isolated membrane patches also provides measurements of electrical currents through single K^+ channels (Hamill et al., 1981). The difficulty in identifying certain K^+ channel subtypes in some tissues using these techniques may be due to their low density, fragility to the isolation procedure, and/or their irreversible rundown (where the activity of the K^+ channels recorded in cell-free patches spontaneously declines over a relatively short period in the absence of the cytosol. Thus, experimental conditions, such as the isolation procedure and recording techniques, can influence the determination of the properties of K^+ channels.

Radioligand binding assays have identified binding sites

for selected K_{ATP}COs, [^{3}H]P1075 (pinacidil analog) and [^{3}H]cromakalim, in smooth muscle preparations (Howlett and Longman, 1992; Quast *et al.*, 1993). [^{3}H]P1075 binding in rat aorta was inhibited by representatives from all chemical families of K_{ATP}COs with potencies that correlated with the potencies obtained in $^{86}Rb^+$ efflux and vasorelaxation studies (Quast *et al.*, 1993). Differences in the data obtained with [^{3}H]P1075 and [^{3}H]cromakalim in smooth muscle preparations, however, suggest the existence of two different binding sites for K_{ATP}COs (Lawson and Hicks, 1993). The use of strips of tissue with intact cells, not membrane preparations, being critical in this assay would suggest that the binding of these K_{ATP}COs is dependent on the functional integrity of the cell (Quast *et al.*, 1993). The development of similar assays for other subtypes of K^+ channel, however, are dependent on the availability of appropriate ligands. Ligand binding studies on expressed cloned K^+ channels (e.g., ROMK1; Ho *et al.*, 1993), which should provide major advances in our understanding of how KCOs interact with the channel, are awaited.

Functional isolated organ preparations have been used extensively in KCO research to determine the mechanical effects due to exposure of tissues to such compounds. Studies in parallel with those techniques described above can demonstrate whether or not the functional responses to KCOs are compatible with and, thereby, a consequence of, K^+ channel opening and K^+ flux. Finally, the effects of KCO are determined in preclinical *in vivo* models predictive of potential therapeutic applications (see Section 4).

2.1. *ATP-Sensitive K^+ Channel Openers*

K_{ATP} channels, which have been studied extensively, initially were identified in cardiac cells and pancreatic β-cells (Noma, 1983; Ashcroft and Ashcroft, 1990). The intracellular concentration of ATP determines the state (open, closed) of the K_{ATP} channel (Edwards and Weston, 1993). Cook and Hales (1984) proposed that K_{ATP} channels in pancreatic β-cells are spontaneously open under normal conditions and, as a result, a basal efflux of K^+ from the cell leads to hyperpolarization of the cell membrane. An increase in the glucose levels evokes a rise in intracellular ATP levels, closing the K_{ATP} channel. The resultant membrane depolarization and subsequent Ca^{2+} influx through voltage-operated Ca^{2+} channels (VOCs) stimulates insulin release. Exogenous compounds, in particular the sulphonylureas (e.g., glibenclamide), by closing K_{ATP} channels, can induce the release of insulin from pancreatic β-cells.

Subsequent studies have shown K_{ATP} channels to exist in virtually all tissues studied, including skeletal muscle, smooth muscle and neuronal cells (Ashcroft and Ashcroft, 1990). Five different types of K_{ATP} channels have been defined as a consequence of potassium selectivity and sensitivity to calcium, intracellular ATP concentration, and pharmacological modulation (Ashcroft and Ashcroft, 1990). Full pharmacological characterization of the putative subtypes of K_{ATP} channels is presently limited (Ashcroft and Ashcroft, 1990; Gopalakrishnan *et al.*, 1993). Of these channels, the Type I, which are blocked by micromolar concentrations of intracellular ATP, have been extensively studied in a number of cell types. This channel, irrespective of location, always demonstrates sensitivity to sulphonylureas; however, the concentrations of these compounds required to block the K_{ATP} channels are tissue-dependent (Edwards and Weston, 1993). In pancreatic β-cells, modulation of insulin release occurs at nanomolar concentrations of glibenclamide, and micromolar concentrations are required in smooth muscle and cardiac preparations, suggesting at least two concentration ranges of activity. Further, the rank order of potency of K_{ATP}COs on Type I K_{ATP} channels is dependent on the cell type being studied (Edwards and Weston, 1993) and, thus, is open to further differentiation on the basis of pharmacological profile. In addition, the current nomenclature does not take into account the results of recent studies (albeit limited) using molecular biology of the cloned K_{ATP} channel (Ho *et al.*, 1993).

Classification of compounds termed K_{ATP}COs has largely been defined as a consequence of their biological effects being sensitive to blockade by sulphonylureas. Potassium channel opening properties, as identified by electrophysiological and efflux studies, sensitive to glibenclamide (or other sulphonylureas) have been demonstrated in a diverse range of synthetic chemical structures (benzothiadiazines [e.g., diazoxide], pyrimidines [e.g., minoxidil], pyridylcyanoguanidines [e.g., pinacidil], nicotinamides [e.g., nicorandil], benzopyrans [e.g., cromakalim] and carbothiamides [e.g., RP 49356]) (Edwards and Weston, 1990; Lawson and Hicks, 1993). Common chemical structural features between benzopyrans, pyridylcyanoguanidines, and carbothiamides have been described (Atwal, 1992). In addition, most of the biological activity resides predominantly in the (−)-enantiomers of cromakalim, pinacidil, and RP 49356.

In functional *in vitro* pharmacology studies, the antagonism by glibenclamide of the effects of K_{ATP}COs on vascular (Eltze, 1989a; Wilson, 1989; Newgreen *et al.*, 1990) and certain nonvascular (Eltze, 1989b; Piper *et al.*, 1990; Edwards *et al.*, 1991) smooth muscle preparations has been described as competitive in nature. Competitive antagonism is inferred by parallel displacements of the K_{ATP}CO-induced relaxant concentration-response curves to the right of controls, without a reduction of the maximum effect to the 'agonist' by the antagonist, and Schild analysis (Arunlakshana and Schild, 1959) yielding a slope not different from unity. The failure of glibenclamide (up to 10 μM) to displace [^{3}H]cromakalim from its binding sites on rat aorta (Howlett and Longman, 1992) does not support a competitive interaction between these two compounds at a single site. Glibenclamide concentration dependently increased the dissociation rate of the [^{3}H]P1075 binding complex on rat aorta (Quast *et al.*, 1993), suggesting that the glibenclamide site is distinct from, but negative allosterically coupled to, the binding site for the openers.

Parallel displacement of concentration-response curves could also be observed with physiological antagonism and, also, if spare channels were available (i.e., the K_{ATP}CO can

evoke a maximal response without the activation of all of the channels in the tissue). The smooth muscle relaxant responses to K_{ATP}COs in the guinea-pig trachea, however, are blocked by glibenclamide in a manner that is not consistent with competitive antagonism (i.e., the maximum effect of the K_{ATP}CO is reduced in the presence of the antagonist) (Nielsen-Kudsk *et al.*, 1990; Berry *et al.*, 1991; Small *et al.*, 1992), but is consistent with a lack of spare channels (i.e., the K_{ATP}CO must activate all channels in the tissue to evoke a maximal response). Thus, glibenclamide appears to interact differently with the channel activated by K_{ATP}COs in respiratory smooth muscle from that in vascular tissue.

In vascular preparations, glibenclamide antagonized the effects of cromakalim competitively, whereas the effects of minoxidil sulphate were antagonized in a noncompetitive manner (Wickenden *et al.*, 1991). These findings led to the suggestion of the existence of different subtypes of K^+ channel sensitive to K_{ATP}COs. In addition, minoxidil sulphate, in contrast to diazoxide and cromakalim, had no effect on $^{86}Rb^+$ efflux in rat aorta, but did increase $^{42}K^+$ efflux (Newgreen *et al.*, 1990; Wickenden *et al.*, 1991). All three K_{ATP}COs increased $^{86}Rb^+$ and $^{42}K^+$ efflux from rat portal vein (Newgreen *et al.*, 1990). Thus, in rat aorta, minoxidil sulphate may open a K^+ channel impermeable to $^{86}Rb^+$ and not recognised by other K_{ATP}COs.

A number of anomalies exist within the pharmacology of the different chemical structures that have been tenuously classified into the family of K_{ATP} openers (Lawson and Hicks, 1993). A common feature responsible for the grouping of these compounds has been the susceptibility of the biological effects to inhibition by sulphonylureas. Nevertheless, the rank order of potency of the inhibitory activity and nature of antagonism by the sulphonylurea is dependent on the cell type and K_{ATP}CO being studied (Longman and Hamilton, 1992; Edwards and Weston, 1993; Lawson and Hicks, 1993). The pharmacological profile of (certain) K_{ATP}COs on the K_{ATP} channel is also dependent upon cell type (e.g., diazoxide is an 'activator' in pancreatic cells, inactive in skeletal muscle cells, and an 'antagonist' in cardiac cells) (Zunkler *et al.*, 1988; Faivre and Findlay, 1989; Weik and Neumcke, 1990). Discrimination between the pharmacology of benzopyran and nonbenzopyran K_{ATP}COs has been identified (Lawson *et al.*, 1992; Randall and Griffith, 1993), where all compounds have exhibited definable KCO profiles. Such findings support the concept that there are differences in the way these agents interact with potassium channels, whichever subtype they may be (Lawson *et al.*, 1992; Lawson and Hicks, 1993).

The ability of glibenclamide to antagonize the actions of this group of compounds led to the suggestion of K_{ATP} channels being the site of action; however, this is still an area of research and discussion. Determination of a common site of action for this group of compounds is complicated by, at least, (1) the existence of subtypes of KATP channels, (2) evidence of these compounds opening several K^+ channels, and (3) effects of glibenclamide on K^+ channels other than K_{ATP} channels.

Using patch-clamp techniques on the rabbit portal vein, Beech *et al.* (1993a,b) found that nucleotide diphosphates (NDPs), such as GDP, are required for K_{ATP} channel opening in the absence of the inhibitory effects of ATP. This property sets them apart from the K_{ATP} channel present in the pancreas and heart. These authors suggested that this K^+ channel in rabbit portal vein should be more correctly termed K_{NDP} to reflect the obligatory role that NDPs play in regulating this particular channel. Together with the recent findings of Kitamura and Kamouchi (1993) that K_{ATP} channels appear to be nonuniform across species in various smooth muscle cells (SMC), evidence suggests that there may be several different types of ATP-, adenine nucleotide-, and glibenclamide-sensitive K_{ATP} channels in various tissues that may be important targets for compounds that can selectively regulate their activity. These findings highlight the complexity of the biological system and the need to fully investigate the specificity of channel/ligand interactions to clearly define the site of action of openers of K_{ATP} channels.

A number of K_{ATP}COs, however, (e.g., pinacidil and nicorandil) possess additional properties (e.g., adenylate or guanylate cyclase stimulation) to that of potassium channel opening that could account, in part, for pharmacological profiles differing from those of the benzopyrans (e.g., cromakalim) and carbothiamides (e.g., RP 49356) (Cook, 1990; Longman and Hamilton, 1992). The situation is further complicated by agents defined as K_{ATP}COs and glibenclamide exhibiting the actions on K^+ channels other than K_{ATP} channels, Ca^{2+} channels and Cl^- currents. Glibenclamide can inhibit delayed rectifier K^+ currents (Reeve *et al.*, 1992; Beech *et al.*, 1993b; Crepel *et al.*, 1993), A type K^+-currents (Sadraei and Beech, 1995), Ca^{2+} channel currents (Bian and Hermsmeyer, 1994; Sadraei and Beech, 1995) and Cystic Fibrosis Transmembrane Regulator-dependent Cl^- current (Sheppard and Welsh, 1992). Cromakalim activated K_{Ca} current (Gelband and McCullough, 1993) and inhibited Ca^{2+} channel current (Okabe *et al.*, 1990) and Cystic Fibrosis Transmembrane Regulator-dependent Cl^- current (Sheppard and Welsh, 1992).

A heterogeneity in the pharmacology of the K_{ATP}COs can be identified, which would suggest a heterogeneity of the K_{CO} sites of action and/or the K^+ channel. Interpretation of data is further complicated by the potential heterogeneity of sulphonylurea-sensitive sites of deactivation that may be independent of the K_{ATP}CO site of action. Further work involving comparative studies with representatives of each chemical family of K_{ATP}COs will assist in the understanding and subclassification of these heterogeneous sites of action. A combined effort of electrophysiology, functional pharmacology, and molecular biology, with a subsequent study of the expressed clone(s), will be required to address the real question of channel selectivity and K_{ATP}CO subclassification. The differences already observed in the pharmacology of K_{ATP}COs are, therefore, important factors to consider in the development of second generation compounds, where tissue or organ selectivity is sought.

2.2. *Calcium-Activated Potassium Channel Openers*

K_{Ca} channels are activated by membrane depolarization and by increases in intracellular calcium (Blatz and Magleby, 1987). Three subtypes of K_{Ca} channels have been described on the basis of their single-channel conductance and sensitivity to specific pharmacological blockers (Cook, 1990). High-conductance or *maxi*-K (BK_{Ca}; 100–250 pS) channels are sensitive to charybdotoxin and iberiotoxin, intermediate-conductance (IK_{Ca}; 18–50 pS) channels are blocked by high concentrations of charybdotoxin, and low- or small-conductance (SK_{Ca}; 10–14 pS) channels are potently blocked by apamin. K_{Ca} channels have been identified in virtually all types of cells, where they almost certainly function to terminate excitatory processes that are triggered or maintained by an increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and/or involve depolarization. As a consequence of such an important physiological role of K_{Ca} channels, the search and recent identification of selective synthetic BK_{Ca} openers has received significant attention.

Two major chemical groups, benzimidazoles (e.g., NS-004, NS-1619) and imidazo(1,2-a)pyrazines (e.g., SCA40), recently have been reported to exhibit BK_{Ca} channel opening properties (Sahlavrolles *et al.*, 1988; Olesen and Watjen, 1992; Laurent *et al.*, 1993; Olesen *et al.*, 1993). NS-004 directly activated the charybdotoxin-sensitive BK_{Ca} channel in rat cerebellar granule cells, in rat GH3 clonal pituitary tumor cells, bovine aortic SMC and guinea-pig tracheal SMC (Olesen and Watjen, 1992; Olesen *et al.*, 1993, 1994a; McKay *et al.*, 1994). NS-1619 directly activated the BK_{Ca} channel in rat ventromedial hypothalamic neurones, bovine aortic SMC, and rat portal vein (Edwards *et al.*, 1994; Sellers and Ashford, 1994; Olesen *et al.*, 1994b). Although NS-1619 failed to modify the K_{ATP} channel in rat ventromedial hypothalamus (Sellers and Ashford, 1994), in the rat portal vein, this compound was reported to inhibit the KV channel over the same concentration range that activated the BK_{Ca} channel (Edwards *et al.*, 1994). NS-004 induced relaxation in guinea-pig trachea, which were sensitive to charybdotoxin and iberiotoxin, but not glibenclamide (K_{ATP} channel), dofetilide (inward rectifier) or apamin (SK_{Ca}), suggesting activation of only BK_{Ca} channels being responsible for this functional response (Lawson *et al.*, 1996). Although NS-004 and NS-1619 activate BK_{Ca} channels, their relaxant effects in rat portal vein and cardioprotective effects probably are due to inhibition of L-type calcium channels (Edwards *et al.*, 1994; Sargent *et al.*, 1993).

In guinea-pig tracheal smooth muscle, SCA40 evoked relaxations that were inhibited by charybdotoxin, suggesting the involvement of BK_{Ca} channels (Laurent *et al.*, 1993). However, SCA40 had no effect on the activity of BK_{Ca} channels in bovine tracheal SMC (Macmillan *et al.*, 1995). Cook *et al.* (1995) reported that relaxant effects of SCA40 in guinea-pig tracheal smooth muscle are due to the inhibition of phosphodiesterase. Activation of K_{Ca} channels in respiratory smooth muscle following β-adrenoceptor stimulation appears to be caused by phosphorylation mediated by a cyclic AMP (cAMP)-dependent protein kinase (Kume *et al.*, 1989). Thus, the K_{Ca} potassium channel opener ($K_{Ca}CO$) profile of SCA 40 may be due to an indirect action on the cAMP pathway.

Dehydroso-vasaponin-I (DHS-I), derived from the medicinal herb *Desmodium adscendens* used therapeutically in the treatment of dysmenorrhea and asthma (Ampofo, 1977), opens charybdotoxin-sensitive K_{Ca} channels in bovine tracheal smooth muscle membranes (McManus *et al.*, 1993). Data regarding the pharmacology of DHS-I is limited and, therefore, it is not known if it is the active factor of the herb and, if so, whether K^+ channels are the primary site of action.

The thiazide diuretic hydrochlorothiazide evokes relaxation in vascular smooth muscle due to the activation of charybdotoxin-sensitive K^+ channels (Calder *et al.*, 1994). These effects have been associated with an increased $^{86}Rb^+$ efflux (Calder *et al.*, 1994) and a decrease in $[Ca^{2+}]_i$ (Pickkers and Hughes, 1995). An increased $^{86}Rb^+$ efflux has also been observed with the thiazide drug cyclothiazide in arterial smooth muscle (Moura and Worcel, 1983). Whether or not thiazides directly act on a K^+ channel remains to be established.

2.3. *Endogenous Openers*

Endogenous openers of potassium channels can be divided into three basic groups: (1) ligands that modulate the gating of potassium channels following interaction with plasmalemma receptors, (2) intracellular second messengers (e.g., inositol trisphosphate, inositol 1,3,4,5 tetrakisphosphate, cAMP), and (3) extracellular substances believed to act directly on the channel. Although it is this latter group that will be the subject of this section, the former groups are of great interest in the understanding of the pathophysiology of disease states and subsequent treatment. In pathologic situations, there may be a defect in the neurotransmitter or receptor (e.g., noradrenaline, 5-hydroxytryptamine [5-HT], acetylcholine, opioid peptides), resulting in decreased activation of the associated K^+ channel. Direct modulation of the K^+ channel through specific agents could provide focused therapy, depending on the distribution of that channel subtype.

Endothelial cells, which line all blood vessels and the cardiac cavity, have a central role in cardiovascular homeostasis, at least in part, through the release of substances that mediate control of vascular tone and cardiac contraction (Shah, 1992). Stimuli of receptors (e.g., acetylcholine, bradykinin) or nonreceptor processes (e.g., electrical field stimulation, calcium ionophore A23187) induce endothelium-dependent hyperpolarization of SMC, leading to vasorelaxation (Garland *et al.*, 1995).

The first endothelium-derived mediator found to act on vascular SMC was prostacyclin (PGI_2), a vasorelaxing cyclooxygenase product (Weksler *et al.*, 1978). The stable analog iloprost, like prostacyclin, evokes a hyperpolarization of SMC through the activation of K^+ channels (Siegel *et al.*, 1990). K_{Ca} channels have been proposed to be the site of action

of iloprost (Boeynaems and Ramboer, 1990); whether this property of iloprost is due to activation of PGI_2 receptors or a direct action on the K^+ channel is still a subject of debate. The development of other stable analogs of PGI_2 that demonstrate therapeutic benefit (e.g., in peripheral arterial occlusive disease, Raynaud's syndrome, cardioprotection, stroke) by the activation of K^+ channels is an active area of research.

Furchgott and Zawadski (1980) demonstrated the importance of the vascular endothelium in mediating the relaxant effect of acetylcholine, leading to the proposal of the existence of endothelium-derived relaxing factor (EDRF). EDRF is believed to be nitric oxide (NO) or a closely related NO-releasing molecule (Palmer *et al.*, 1987). EDRF-induced smooth muscle relaxation is mediated, at least in part, by the haem-dependent activation of guanylate cyclase, with the subsequent generation of cyclic GMP (cGMP) (Furchgott and Jothianandan, 1983; Rapoport and Murad, 1983). Before the discovery of the role of the endothelium in vasodilation, Kuriyama and Suzuki (1978) had observed that acetylcholine hyperpolarized SMC by increasing the membrane permeability to K^+ ions. Although NO has been reported to change smooth muscle membrane potential (Tare *et al.*, 1990) and directly activate K_{Ca} in SMC (Bolotina *et al.*, 1994), the contribution of these effects to endothelial-dependent relaxation relative to direct stimulation of guanylate cyclase is unclear. Taylor and Weston (1988) subsequently suggested that an additional factor to EDRF, which could cause vasorelaxation by increasing the membrane potential of the muscle cells, was released from the endothelium by acetylcholine. To distinguish this factor from EDRF, it was termed endothelium-derived hyperpolarizing factor (EDHF). Although evidence has been presented to support the hypothesis that EDHF is a diffusible factor released from the endothelium, an electrotonic spread of hyperpolarization between the endothelial and SMC cannot be eliminated at this stage (Garland *et al.*, 1995). The chemical identity of EDHF remains unresolved; however, a number of candidates (e.g., prostanoids) have been eliminated (Garland *et al.*, 1995). The proposal that EDHF may be a cytochrome P450-derived arachidonic acid metabolite, such as epoxyeicosatrienoic acids, requires confirmation (Gebremedhin *et al.*, 1992; Hecker *et al.*, 1994). Experiments to advance the identification of EDHF may be complicated by the existence of a family of factors, as opposed to a single substance.

The effects of EDHF are associated with the efflux of K^+ ions from SMC (Chen and Suzuki, 1989), but the channel(s) involved have not been definitively characterized. The majority of studies have found that glibenclamide blocks the change in membrane potential, suggesting the involvement of K_{ATP} channels (Chen and Cheung, 1992; Eckman *et al.*, 1992; Garland and McPherson, 1992; Adeagbo and Triggle, 1993). In certain vascular beds, however, K_{Ca} channels have also been proposed to be the site of action of EDHF (Adeagbo and Triggle, 1993). The existence of more than one EDHF may also complicate the identification of the site of action.

Physiologically, the action of a hyperpolarizing factor that is distinct from NO appears to predominate in the mediation of endothelial-dependent smooth muscle relaxation in small resistance arteries (Garland *et al.*, 1995). In large arteries, both NO and EDHF appear to contribute to relaxation, with NO being dominant under normal circumstances. Thus, EDHF and the K^+ channel it activates may be of primary importance in the regulation of vascular resistance. The clinical importance of EDHF in disease aetiology will only be appreciated when the chemical identity is established and/or the K^+ channel(s) activated is characterized.

The endothelins are a family of 21-amino acid vasoactive peptides, of which endothelin-1 (ET-1) has been reported as the most potent vasoconstrictor known (Randall, 1991). ET-1, however, has also been demonstrated to modulate K_{ATP} channels in both *in vitro* cell culture (Inoue *et al.*, 1990) and *in vivo* studies (Hasunuma *et al.*, 1990; Lippton *et al.*, 1991). As a consequence of this property, ET-1 preferentially evokes vasodilation in certain vascular beds (e.g., pulmonary; Lippton *et al.*, 1991). In addition, the vascular effects of benzopyran, but not carbothiamide and pyridylcyanoguanidine, K_{ATP}COs (see Section 2.1) were modified by ET-1, suggesting that BRL-38227 and ET-1 have affinity for a common site on the K_{ATP} channel (Lawson *et al.*, 1992). This hypothesis is supported by the finding that BRL-38227 inhibits binding of [^{125}I]ET-1 to rat cardiac membranes (Waugh *et al.*, 1992). Further, ET-1 evokes membrane hyperpolarization in rat aortic SMC (Van Renterghem *et al.*, 1988), rat glioma C6-BU-1 cells (Gleason *et al.*, 1991), porcine coronary artery cells (Hu *et al.*, 1991), rat gastric longitudinal and circular smooth muscle (Fulginiti *et al.*, 1993) and guinea-pig taenia coli (Usune *et al.*, 1991) through the activation of charybdotoxin-sensitive K_{Ca} channels. In contrast, ET-1 also exhibits antagonistic properties at both K_{ATP} (Miyoshi *et al.*, 1992) and BK_{Ca} (Hu *et al.*, 1991) channels.

Consequently, ET-1 can directly modulate K^+ channels having affinity for both K_{ATP} and K_{Ca} channels. The (patho)physiological role of ET-1-induced K^+ channel activation remains to be established. The recent advances in the identification of selective antagonists will allow the elucidation of the involvement, if any, of ET-receptors in this action of the peptide.

Interestingly, ET-1 and ET-3 can also evoke an indirect endothelium-dependent hyperpolarization in rat mesenteric smooth muscle (Nakashima and Vanhoutte, 1993a), but not in canine coronary artery (Nakashima and Vanhoutte, 1993b).

Finally, a variety of endogenous polypeptides have been proposed to exhibit K^+ channel opening properties. The endogenous vasodilator peptides, vasoactive intestinal peptide and calcitonin gene-related peptide (CGRP), activate K_{ATP} channels in vascular SMC, leading to hyperpolarization and tissue relaxation (Standen *et al.*, 1989; Nelson *et al.*, 1990). CGRP is a polypeptide located in neurones that form a close association with both central and peripheral blood vessels (Bevan and Brayden, 1986). The CGRP-induced relaxations of some blood vessels (e.g., rat aorta) are endothelium-dependent; thus, the role of endothelium-

derived factors in the observed hyperpolarization has been proposed (Grace *et al.*, 1987; Nelson *et al.*, 1990). Galanin and somatostatin, hyperglycemic hormones, activate K_{ATP} channels in insulin secreting pancreatic β-cells (De Weille *et al.*, 1988, 1989). The block of anoxia-induced depolarization of hippocampal CA3 neurones and glutamate release inhibition by galanin is consistent with K^+ channel opening (Ben-Ari, 1990). However, not all reports support the conclusion of K^+ channel activation as a property of vasoactive intestinal peptide, CGRP, galanin, and somatostatin; further work is required to establish the role of K^+ channels in their physiological profiles.

2.4. *Others*

1,1-Dimethyl-4-phenylpiperazinium (DMPP), a selective nicotinic receptor stimulant, has shown activity consistent with K^+ channel activation in the isolated tunica muscularis mucosae of the rat oesophagus (Adeagbo *et al.*, 1993). Neither apamin nor glyburide modified the DMPP-induced relaxation. Whether this was a direct action of DMPP on a K^+ channel or a nicotinic-cholinoceptor-linked K^+ channel requires elucidation.

3. MECHANISM(S) OF ACTION

The standard criteria that initially identified a compound as a KCOs has been its ability to relax an *in vitro* smooth muscle preparation contracted with low, but not high, concentrations of extracellular K^+ ions (Weir and Weston, 1986; Bray *et al.*, 1987; Hamilton and Weston, 1989; Lawson and Cavero, 1989). The principal determinant for inhibition or reversal of smooth muscle contraction by a KCO is a reduction in the free cytosolic Ca^{2+} concentration. The mechanism(s) involved in the production of smooth muscle relaxation by K^+ channel openers, especially K_{ATP}COs, has been (and still is) a major subject of debate. To date, information regarding mechanism(s) of action primarily has been obtained from studying K_{ATP}COs, especially cromakalim and other benzopyrans. Investigations with other classes of KCOs (e.g., K_{Ca}COs) that will demonstrate whether or not mechanism(s) of action are common between the different K^+ channels and how they are activated by different ligands, are awaited. The mechanism of action involved in a given response may not only depend on the subtype of K^+ channel, but also how the KCO interacts with the channel to evoke that effect (Lawson *et al.*, 1992; Randall and Griffith, 1993).

Although some mechanism(s) are universal in different excitable and nonexcitable cells, not all processes will be applicable to all types of tissue. The majority of data has been obtained from SMC and requires confirmation in other cells.

3.1. *Hyperpolarization*

The opening of K^+ channels by these compounds and subsequent efflux of K^+ ions from the cytosol leads to membrane repolarization and/or hyperpolarization (Cook, 1990; Edwards and Weston, 1990; Robertson and Steinberg, 1990; Longman and Hamilton, 1992). This change in membrane potential is followed by a reduction in cytosolic free Ca^{2+} and/or an inhibition of mechanisms producing increases in cytosolic free Ca^{2+}. The outcome of these effects is a reduction in membrane and cell excitability, resulting in a greater cellular resistance to activation by excitatory stimuli.

In smooth muscle preparations, the relaxant actions of K_{ATP}COs have been accompanied by an increase in negativity of the resting membrane potential (hyperpolarization) towards the calculated E_K, together with an outward current of K^+ ions (Hamilton and Weston, 1989).

An excellent correlation exists between the potencies of the K_{ATP}COs for stimulation of $^{86}Rb^+$ efflux and vasorelaxation in rat aorta and portal vein *in vitro* preparations (Quast *et al.*, 1992), supporting the hypothesis that the functional responses rely on the opening of plasmalemma K^+ channels in vascular smooth muscle.

Although, initially, it was assumed that the hyperpolarization caused by K^+ efflux produced closure of VOCs, preventing depolarization-induced Ca^{2+} entry into the cell, other mechanisms, in light of recent evidence, may also contribute to the effects produced by K^+ channel openers (see Sections 3.2, 3.3 and 3.4). Evidence that cromakalim-evoked increase in $^{86}Rb^+$ efflux or hyperpolarization of vascular smooth muscle tissue was not influenced by lanthanum or the Ca^{2+} antagonist nifedipine, is indicative that the action of the K_{ATP}COs is not dependent upon modifying the influx of external Ca^{2+} ions (Coldwell and Howlett, 1988; Southerton *et al.*, 1988).

Vasorelaxant effects of K_{ATP}COs have been reported that are independent of membrane hyperpolarization or ion efflux (Hamilton *et al.*, 1986; Quast and Baumlin, 1988; Greenwood and Weston, 1993). Such findings suggest that these drugs can exert a response through mechanisms other than the opening of K^+ channels. The possibility that K_{ATP}COs do not interact directly with an ion channel but, rather, for example, with an enzyme system involved in intracellular phosphorylation, provides a novel explanation for some of the apparently anomalous effects of these agents (Edwards and Weston, 1994).

3.2. *Intracellular Ca^{2+} Stores*

Experimental evidence suggests that K_{ATP}COs may also have a direct effect on intracellular stores. Cromakalim evoked a contractile response in rabbit aorta bathed in a Ca^{2+}-free solution, which may be related to effects on intracellular Ca^{2+} stores (Duty and Weston, 1992). Using rabbit cultured tracheal SMC, Chopra *et al.* (1992) demonstrated that cromakalim reduced the uptake into and inhibited the release of $^{45}Ca^{2+}$ from the sarcoplasmic reticulum. These findings support those obtained from vascular smooth muscle, where contractile responses to noradrenaline, dependent on intracellular calcium stores, were attenuated by cromakalim (Bray *et al.*, 1991).

In contrast, the effect of cromakalim on rat pulmonary artery did not appear to involve an action on Ca^{2+}-release from internal stores (Savineau and Marthan, 1993).

3.3. *Inositol Phosphate Cascade*

BRL 38227 inhibited Ca^{2+} release from intracellular stores of rabbit isolated mesenteric artery due to an action on noradrenaline-stimulated 1,4,5-inositol trisphosphate production (Ito *et al.*, 1991). This effect was sensitive to glibenclamide and high (128 mM) extracellular KCl. Hyperpolarization of the plasma membrane of canine coronary artery by $K_{ATP}COs$ has also been associated with an inhibition of the production of 1,4,5-inositol trisphosphate and, hence, Ca^{2+} release from intracellular stores (Yamagishi *et al.*, 1992). Interestingly, $K_{ATP}COs$ have shown no effect upon phosphatidyl inositol turnover in brain tissue (Coldwell and Howlett, 1988).

3.4. *Ca^{2+} Sensitivity*

The hyperpolarization by $K_{ATP}COs$ (e.g., levcromakalim) may also be linked with a reduction in the sensitivity of the contractile elements of vascular smooth muscle to Ca^{2+} (Okada *et al.*, 1993). Cromakalim, however, has no direct effect on the contractile proteins of 'skinned' trachealis muscle (Allen *et al.*, 1986).

3.5. *Others*

Cromakalim has been reported to stimulate the Na-K pump in human and canine mesenteric arteries through an elevation in intracellular Na^+ (Hong *et al.*, 1993). In rat aorta, cromakalim appears to be absent of effects on the Na-K pump (Cavero *et al.*, 1987).

The $K_{ATP}COs$, cromakalim, and RP-49356 failed to increase cAMP or cGMP in SMC (Southerton *et al.*, 1988; Nakajima *et al.*, 1989), or to potentiate the effects of forskolin in airway smooth muscle preparations (Berry *et al.*, 1991; Murray *et al.*, 1990). Pinacidil and cromakalim do not appear to be inhibitors of phosphodiesterase activity in airway smooth muscle (Berry *et al.*, 1991; Ho *et al.*, 1990). Guanine nucleotide-binding proteins (G-proteins) are known to provide a link between membrane receptors and intracellular events, and the possibility exists that G-proteins may modulate the activity of a K^+ channel sensitive to $K_{ATP}COs$. Results with pertussis or cholera toxin, however, suggest that the corresponding G-proteins are not involved in the actions of cromakalim in vascular smooth muscle (Longman and Hamilton, 1992).

Therefore, the involvement of intracellular Ca^{2+} stores in the responses to $K_{ATP}COs$ may be species- and/or tissue-dependent. Further studies are required to determine whether or not this is a property unique to cromakalim (or benzopyrans) and if it is a direct effect or a consequence of hyperpolarization. Thus, the mode of action of K^+ channel openers may not be as simple as first thought and more research effort is required in this area.

4. THERAPEUTIC TARGETS AND POTENTIAL

Theoretically, at least, the general decrease in the excitability of cells that follows K^+ channel opening infers a broad clinical potential for drugs with this property in a number of pathologic conditions.

4.1. *Cardiovascular System*

The preclinical profile of $K_{ATP}COs$ clearly supports a clinical potential for their use in vascular pathologies that require a decrease in peripheral vascular resistance, an inhibition of excessive vasoconstriction, and/or a prolongation of myocardial tissue viability, while undergoing transient oxygen deficiency.

4.1.1. Vascular. The pharmacological profiles of $K_{ATP}COs$ in vascular smooth muscle tissues and *in vivo* models of vascular disorders (e.g., hypertension, peripheral vascular disease, angina) have been reviewed extensively (Edwards and Weston, 1990; Richer *et al.*, 1990; Longman and Hamilton, 1992; Edwards and Weston, 1995). In a variety of vascular tissues from a range of species, $K_{ATP}COs$ display the ability to relax smooth muscle by inhibiting both spontaneous tone and/or spasmogen-induced contraction. Arterial smooth muscle tone, of which K^+ channels in the SMC are important regulators, is the main determinant of peripheral vascular resistance and blood pressure. Functional defects of K^+ channels may lead to vasoconstriction or compromise the ability of an artery to dilate in pathologic conditions of the vasculature, such as vasospasm, hypertension, ischemia, hypotension following sepsis, and diabetes.

Activation of K_{ATP} channels, which respond to the metabolic state of the cell, may be involved in arterial dilation in reactive hyperemia, septic shock, ischemia, and hypoxia (Nelson, 1993). The involvement of K_{ATP} channels in these clinical conditions may not be exclusive; however, the absence of selective agents of other K^+ channels presently limits full characterization of the aetiology of such diseases.

$K_{ATP}COs$ can lower both normal and experimentally elevated blood pressure (Edwards and Weston, 1990; Richer *et al.*, 1990; Longman and Hamilton, 1992). The mechanism of this effect is an actively mediated decrease in total peripheral vascular resistance. Therapeutic experience with pinacidil, diazoxide, and minoxidil indicates that they also reduce elevated blood pressure in patients (Gross, 1977; Oates *et al.*, 1977; Ahnfelt-Rønne, 1988). Hypertensive patients treated with pinacidil (10–25 mg b.i.d.) showed a marked fall in total peripheral resistance, the short duration of which was controlled through the development of sustained-release formulations (Carlsen *et al.*, 1983, 1985). The antihypertensive effects of pinacidil are accompanied by an initial reflex tachycardia and by weight gain in approximately 40% of patients. Although the antihypertensive effects of diazoxide have been known for many years (Gross, 1977; Oates *et al.*, 1977), increased blood glucose levels has restricted its use to hypertensive emergencies. The extent to which the antihypertensive effects of diazoxide result from K^+ channel

opening action is still unknown, as evidence of additional effects have been observed (Newgreen *et al.*, 1990). The relatively high incidence of fluid retention has severely restricted the clinical use of minoxidil (Gross, 1977; Oates *et al.*, 1977). The K^+ channel opening profile of minoxidil, however, is different from that of diazoxide and cromakalim, whereby the involvement of a minoxidil-selective channel has been proposed (Newgreen *et al.*, 1990; Lawson and Hicks, 1993).

Clinical experience with cromakalim is less extensive than for the above K_{ATP}COs, and there are no published reports of clinical trials with the other K_{ATP}COs (e.g., RP 49356, Ro 31-6930, EMD 52692, KRN 2391). In mild to moderate hypertension, cromakalim (0.5–1.5 mg p.o.) lowered systolic and diastolic blood pressure following a single oral dose (Vanden Burg *et al.*, 1986; Singer *et al.*, 1989) or chronic once-daily administration (Eckl and Greb, 1987; Lebel *et al.*, 1989; Vanden Burg *et al.*, 1987), with no such effects in a parallel normotensive group or when placebo was administered. As a direct vasodilator, the use of KCOs as monotherapy to reduce blood pressure can produce a series of undesirable effects (e.g., tachycardia, headache, flushing, increase in renin, aldosterone, and catecholamine secretion, and sodium and water retention (Gluck *et al.*, 1987; Lijnen *et al.*, 1989) that are not acceptable in clinical practice. K_{ATP}COs, however, could become useful antihypertensive therapy if appropriately formulated and coprescribed with selected agents to reduce or prevent the undesirable events. Due to apparent cardioprotective properties (see Section 4.1.2), small doses of K_{ATP}COs could be used to provide myocardial protection to hypertensive patients, an area where current drugs do not appear to substantially reduce cardiovascular mortality and morbidity (Escande and Cavero, 1992; Cavero and Premmereur, 1994; Grover, 1994a,b).

In preclinical studies, K_{ATP}COs relaxed coronary conductance arteries, increased selectively coronary blood flow and antagonized the vasoconstrictor activity of a large number of excitatory stimuli (Longman and Hamilton, 1992). If similar findings are reproduced in human subjects, K_{ATP}COs would have antianginal activity at doses that do not provoke undesirable, reflex-mediated activation of the sympathetic system, which is a deleterious physiological reaction for myocardium frankly ischemic or lacking a safety margin of blood flow reserve. Thus, these agents demonstrate properties desirable to improve oxygen delivery and also reduce oxygen consumption within ischemic regions of patients with transient and chronic heart disease (e.g., angina pectoris). Reports on the effects of KCOs in angina pectoris, however, are restricted primarily to trials in which nicorandil was used (Holzmann, 1983; Kinoshita and Sakai, 1990). The clinical benefits of nicorandil probably result both from K^+ channel opening properties and its ability to stimulate smooth muscle guanylate cyclase (Hamilton and Weston, 1989). Cromakalim may also be beneficial in the treatment of angina pectoris (Thomas *et al.*, 1990).

One therapeutic approach to congestive heart failure, although it is of a symptomatic nature, is to reduce peripheral vascular resistance, a property demonstrated by K_{ATP}COs (Gopalakrishnan and Triggle, 1990). In an *in vivo* rat cardiac failure model, down-regulation of ventricular K_{ATP} channel density, together with 1,4-dihydropyridine-sensitive Ca^{2+} channels and β-adrenoceptor densities, was observed, and these changes have been implicated in this pathophysiological condition (Gopalakrishnan *et al.*, 1990). No clinical data for the effectiveness of KCOs in the treatment of congestive heart failure has been published.

Activation of K^+ channels can improve the energy metabolism and the mechanical performance of skeletal muscles suffering oxygen deficiency (Cook and Chapman, 1993). This is achieved partly by a selective dilation of collateral vessels supplying the ischemic skeletal muscle and, partly, by a better utilization of high energy phosphates. In rat skeletal muscle, Angersbach and Nicholson (1988) demonstrated that K_{ATP}COs, but not Ca^{2+} antagonists or hydralazine, selectively increased blood flow to collateral vessels in a previously ischemic limb, despite a reduction in basal diastolic blood pressure. These mechanisms are evidently of therapeutic potential for treating patients with peripheral vascular disease, a disabling old-age disorder characterized by poor blood supply to the limbs due mostly to atherosclerosis. The role of K^+ channel activation in the beneficial effects of iloprost (see Section 2.3; Pessi *et al.*, 1986; Muller Buhl *et al.*, 1987; Oberender *et al.*, 1989) in peripheral arterial occlusive disease still remains to be determined.

Cromakalim (10 μM) increased $^{86}Rb^+$ efflux in control cultured arterial SMC, but was without effect in cholesterol-enriched SMC (Tulenko and Bialecki, 1989). Cholesterol enrichment of SMC membranes can severely influence the cellular responses to cromakalim. Thus, the benefit of K_{ATP}COs in clinical conditions associated with excess lipids may be questionable.

K_{Ca} channels appear to play a fundamental role in regulating the degree of intrinsic tone in resistance arteries (Nelson, 1993) and, as such, help regulate arterial responses to pressure and vasoconstrictors. Therefore, activation of these channels should contribute to vasodilation. Defects in K_{Ca} channels could lead to, or contribute to, pathologic conditions that are characterized by highly constricted arteries. Thus, the development of activators may be useful in the treatment of, for example, coronary or cerebral vasospasm. Although NS-004 and NS-1619 activate BK_{Ca} channels (see Section 2.2), their relaxant effects in rat portal vein (Edwards *et al.*, 1994) and cardioprotective effects (Sargent *et al.*, 1993) are probably due to inhibition of L-type calcium channels.

As blood flow increases through a conduit artery, the vessel dilates (Hilton, 1959) by an endothelium-dependent mechanism (Hull *et al.*, 1986; Pohl *et al.*, 1986). Flow in rabbit isolated iliac arteries appears to activate a charybdotoxin-sensitive K^+ channel on the endothelial cell membrane that leads to the release of NO (Cooke *et al.*, 1991). Neither glibenclamide (K_{ATP} channel blocker) nor apamin (SK_{Ca} channel blocker) had any effect on the flow-mediated vasodilation. Thus, in the regulation of arterial tone, BK_{Ca} channels act as the transducer of the flow stimulus, whereas NO is the effector of the vasodilation.

Endothelium-dependent vasodilations have also been associated with the activation of K_{ATP} channels. The cromakalim- and pinacidil-induced dilation of canine large coronary arteries *in vivo*, an indirect flow-mediated effect, are entirely dependent on the endothelium (Drieu La Rochelle *et al.*, 1992; Ghaleb *et al.*, 1995). The K_{ATP}CO, levcromakalim, was more potent as a vasorelaxant in rat aortic ring preparation with endothelium than in denuded tissues, an effect that involved NO (Lawson *et al.*, 1993b).

In porcine aortic endothelial cells, pinacidil and cromakalim elevated $[Ca^{2+}]_i$ by inducing membrane hyperpolarization following K^+ channel activation (Luckhoff and Busse, 1990), an effect that can promote Ca^{2+}-dependent formation of EDRF. Pinacidil also opened K_{ATP} channels in both rat aorta and brain microvascular endothelial cells (Janigro *et al.*, 1993). These findings suggest that K_{ATP} channels may play a role in the regulation of endothelial cell resting membrane potential, for example, during impaired energy supply and, therefore, modulate release of endothelium-derived vasoactive factors and blood flow.

4.1.2. Cardiac. K^+ channel opening properties are desirable for therapeutic agents aimed at treating patients with transient and chronic heart diseases. K_{ATP}COs have demonstrated cardioprotective effects as a consequence of improving oxygen delivery and reducing oxygen consumption within the ischemic region (Escande and Cavero, 1992; Grover, 1994a,b; Yao and Gross, 1994; Grover *et al.*, 1995). Both antiarrhythmic and proarrhythmic properties have been reported in K_{ATP}COs, leading to the safety of these drugs being a major subject of discussion (Carlsson *et al.*, 1991; Tosaki *et al.*, 1993; Black and Lucchesi, 1994; D'Alonzo and Grover, 1994; Wilde, 1994). Although there appear to be good theoretical arguments as to why K_{ATP}COs may be of use in the treatment of some arrhythmias and in ischemic heart disease, there are major hurdles to overcome.

Clinical evidence to establish the benefits of K_{ATP}COs as treatments of patients with heart disease is awaited. Therefore, only the basic concepts of K_{ATP}CO-induced beneficial or undesirable cardiac effects will be outlined.

Cardiac K_{ATP} channels have been shown to open in response to ischemia (Kantor *et al.*, 1990). The depletion of ATP in the myocardium and subsequent opening of K_{ATP} channels may lead to a rapid reduction in contractility of the ischemic myocardium to protect against further ischemic injury. In support of this hypothesis, cromakalim, RP 52891 (aprikalim), and pinacidil, in animal models, cause a glibenclamide-sensitive reduction in the severity of ischemic/reperfusion injury of the myocardium (Richer *et al.*, 1990; Auchampach *et al.*, 1992; Escande and Cavero, 1992; Grover, 1994a,b). Thus, K_{ATP}COs play a cardioprotective role, whereas glibenclamide worsens myocardial stunning (Auchampach *et al.*, 1992). Analogies have been observed between the cardioprotection conferred by K_{ATP}COs and ischemic preconditioning (i.e., increased tolerance of cardiac myocytes to an ordinarily lethal ischemic insult, achieved by an initial brief exposure to ischemia), for example, both are sensitive to glibenclamide blockade (Gross and Auchampach, 1992). Thus, therapy with K_{ATP}COs may afford a permanent 'chemical preconditioning' that confers on the heart the ability to better withstand transient oxygen deprivation and, consequently, to suffer less tissue damage during acute myocardial infarction.

Whether K_{ATP}COs exert their beneficial effects on the ischemic heart by a direct (myocardial) or indirect (vascular) action remains to be determined. Studies with U-89,232 (cromakalim analog; Toombs *et al.*, 1992) and BMS 180448 (pinacidil analog; Grover *et al.*, 1995), K_{ATP}COs devoid of vascular effects demonstrated cardioprotection against ischemia in animal models greater than that observed with cromakalim. This would suggest that there is a direct myocardial action of these second generation K_{ATP}COs that will provide an opportunity to explore the cardioprotection of such agents without possible complication (e.g., hypotension, coronary steal). The mechanism of tissue selectivity is not clear, but it may be related to the existence of K_{ATP}CO 'receptor' subtypes in different tissues (Atwal, 1994). Similar concepts of subtypes of the site(s) of action of K_{ATP}COs in SMC previously have been proposed in SMC (Piper *et al.*, 1990; Wickenden *et al.*, 1991; Lawson and Hicks, 1993).

Although K_{ATP} channel opening in response to ischemia may offer a cardioprotective mechanism, there is another consequence. The resulting increase in K^+ efflux shortens the action potential duration and contributes to the extracellular K^+ accumulation observed during an ischemic episode (Coetzee, 1992). These changes in conductance and extracellular K^+ have been hypothesized to be responsible for ischemia-induced arrhythmias. K_{ATP}COs have demonstrated arrhythmogenic properties in certain animal studies (Chi *et al.*, 1990; Tosaki *et al.*, 1992; De La Coussaye *et al.*, 1993). However, although K_{ATP}COs may be contraindicated in some types of arrhythmia, they may be of use in the treatment of certain other types of arrhythmia resulting from a repolarization defect (Antzelevitch and Di Diego, 1992). K_{ATP}COs have been shown to suppress rhythm abnormalities related to delayed repolarization and early afterdepolarizations in anaesthetised rabbits (Carlsson *et al.*, 1991).

4.1.3. Blood. The K_{ATP}COs, cromakalim, celikalim, and pinacidil, inhibited white thrombus formation in a rabbit arteriovenous shunt model, although they had no effect on human platelet aggregation (Pareilunas-Hoffman *et al.*, 1994). Antithrombotic activity of K_{ATP}COs *in vivo* may be related to beneficial effects on blood rheology and reduced red blood cell deformability.

4.2. *Respiratory System*

Administration of cromakalim and other K_{ATP}COs to conscious (oral or inhalation route) or anaesthetized (oral, inhalation or i.v. route) guinea-pigs protects against histamine-, 5-HT, or (in sensitized animals) ovalbumin induced bronchoconstriction (Raeburn and Karlsson, 1991). In the anaesthetised animal (Konzett-Rossler model, where protective cardiovascular reflexes are inhibited), a reduction in

diastolic blood pressure was observed following oral or i.v. administration of $K_{ATP}COs$. Bronchodilation, however, could be achieved at doses of cromakalim not reducing mean arterial blood pressure in experiments where the $K_{ATP}CO$ was administered by inhalation; thus, demonstrating selectivity (Bowring et al., 1991; Raeburn and Karlsson, 1991; Bowring et al., 1993; Arch et al., 1994). Respiratory dynamics measurements in anaesthetised guinea-pigs revealed that the $K_{ATP}COs$, cromakalim and BRL 55834, resembled theophylline by eliciting similar inhibition of histamine-induced increase in airways resistance and decrease in lung compliance, and salbutamol, a β-agonist, was more effective against resistance than compliance (Bowring et al., 1991). Therefore, $K_{ATP}COs$, compared to β-agonists are more effective dilators of small airways (where constriction decreases compliance) for identical large airways effects (where constriction increases resistance). Reports have implicated the activation of K_{Ca} channels in the relaxant responses of respiratory smooth muscle to β-agonists (Kume et al., 1989; Jones et al., 1990). Thus, these findings may be suggestive of the distribution of K_{ATP} and K_{Ca} channels within the smooth muscle of the respiratory system. The clinical relevance of such a hypothesis is, as yet, unclear.

$K_{ATP}COs$ can inhibit neurotransmitter release from cholinergic and nonadrenergic, noncholinergic excitatory neurones (NANCe) in guinea-pig lung *in vitro* and *in vivo* (Raeburn and Karlsson, 1991; Small et al., 1992). These neural effects of $K_{ATP}COs$ may be very relevant to potential treatment of asthma because, not only does bronchoconstriction frequently have a significant parasympathetic component, but also neurogenic inflammation of the lung may contribute to the pathology of asthma (Barnes et al., 1991). The main evidence for an effect on neurotransmitter release is that $K_{ATP}COs$ are far more effective at inhibiting cholinergically or NANCe-mediated bronchoconstriction or mucus secretion elicited by stimulating neurotransmitter release than when the relevant neurotransmitter is supplied directly (Ichinose and Barnes, 1990; Burka et al., 1991; Raeburn and Karlsson, 1991; Small et al., 1992). A prejunctional site of action has been proposed for the inhibition of peptidergic excitatory neurotransmitter release due to $K_{ATP}COs$ inhibiting the NANCe neuroeffector transmission at concentrations slightly lower than those causing relaxation of airways smooth muscle. Interestingly, the $K_{ATP}COs$ do not seem to interfere with NANC inhibitory neuroeffector transmission in the lung (Burka et al., 1991). Nielsen-Kudsk et al. (1994) demonstrated that, like cromakalim and pinacidil, terbutaline- (β-agonist), theophylline- (xanthine), and verapamil- (Ca^{2+} antagonist) induced inhibition of NANCe neurotransmission in guinea-pig bronchi involved a prejunctional site.

The inhibition by cromakalim of electrically evoked [^{3}H]-acetylcholine release in rat isolated trachea has been suggested to be an epithelium-dependent mechanism (Wessler et al., 1993). This effect was only observed in tube preparations, where the mucosal/submucosal environment would be better preserved, and not in trachea opened longitudinally.

$K_{ATP}COs$, BRL 38227 and YM-934, inhibited plasma leakage in trachea, main bronchi, and central and peripheral intrapulmonary airways evoked by stimulation of vagal nerves in guinea pigs (Lei et al., 1993; Ishikawa et al., 1994). These compounds had no effect on exogenously administered Substance P-induced plasma leakage. Thus, cromakalim and YM-934 inhibit airway neurogenic inflammation by modulating the release of neuropeptides from the sensory nerve endings, and the inhibitory effect can be attributed to the KCO activity.

$K_{ATP}COs$ can reduce obstruction to airflow by suppressing hyperreactivity of intact airways in animals, with doses that are insufficient to relax airway smooth muscle *in situ* in normal animals (Chapman et al., 1991; Paciorek et al., 1992; Morley, 1994). Hence, the potency of $K_{ATP}COs$ as inhibitors of bronchospasm is greater in hyperreactive than normal animals. An almost universal characteristic of asthmatics is that their airways are hyperresponsive to a wide range of physiological and pharmacological stimuli (Smith, 1992). The causes of airways hyperresponsiveness in humans are not well-defined, although several animal models have been developed to emulate this response. In general, however, the degree of hyperresponsiveness achieved in animals is less than that in humans (Smith, 1989). Compounds that open K^+ channels and impair expression of airway hyperreactivity in the absence of direct smooth muscle spasmolysis will provide a novel approach to symptomatic therapy in asthma (Morley, 1994).

In general, the direct relaxant properties of $K_{ATP}COs$ such as cromakalim have been assessed predominantly in guinea-pig isolated trachealis muscle (Raeburn and Karlsson, 1991, Longman and Hamilton, 1992; Small et al., 1992). This tissue has also been used for ion flux studies and for intracellular recording of change in membrane potential. $K_{ATP}COs$ inhibit contractions to or reverse precontractions to a variety of spasmogens in guinea-pig tracheal preparations. However, in interaction studies, the smooth muscle relaxant responses to $K_{ATP}COs$ in the guinea-pig trachea are blocked by glibenclamide in a manner that is not consistent with competitive antagonism (i.e., the maximum effect of the $K_{ATP}CO$ is reduced in the presence of the antagonist [Berry et al., 1991; Nielsen-Kudsk et al., 1990]), but is consistent with a lack of spare channels (i.e., the $K_{ATP}CO$ must activate all channels in the tissue to evoke a maximal response; see Section 2.1). The antagonism by glibenclamide of the effects of $K_{ATP}COs$ in functional *in vitro* pharmacology studies on vascular smooth muscle and certain nonvascular smooth muscle preparations has been described as competitive in nature (see Section 2.1).

The lack of competitive interaction between glibenclamide and the $K_{ATP}COs$ is indicative of the involvement of more than one mechanism in the relaxant effect of the latter. This is consistent with the conclusion that relaxations of tracheal smooth muscle to BRL 55834 (a benzopyran $K_{ATP}CO$) is mediated by, at least, a glibenclamide-sensitive and a glibenclamide-resistant K^+ channel (Lawson et al., 1993a; Edwards et al., 1995). In addition, BRL 55834 has

been reported to activate an ATP- and glibenclamide-sensitive K^+ channel and, at higher concentrations, a large conductance charybdotoxin-sensitive Ca^{2+}-activated K^+ channel in bovine trachealis SMC (Ward *et al.*, 1992).

Cromakalim and levcromakalim have demonstrated relaxant effects in human bronchial smooth muscle (Taylor *et al.*, 1992; Black *et al.*, 1990). Differences (primarily in potency) from the findings obtained in guinea-pig preparations suggested that the guinea pig is not a good predictor of the inhibitory response of K_{ATP}COs in humans.

Clinical trial of cromakalim in patients with nocturnal asthma showed that a single (0.5 mg) or repeat (0.25, 0.5 mg) oral dose administered at 11:00 p.m. could attenuate the dip in lung function measured at 6:00 a.m. the following morning (Williams et al., 1990). The predicted peak plasma concentration of cromakalim in these studies was about 5-fold less than its threshold concentration for relaxation of tone in human bronchi (Taylor *et al.*, 1992). Studies in animal models prior and subsequent to these findings suggest that the positive results are due to actions other than just relaxation of the bronchial smooth muscle (Longman and Hamilton, 1992; Small *et al.*, 1992; Morley, 1994). The efficacy of cromakalim may not involve the direct relaxation of airways smooth muscle, but is due to an influence on neural mechanisms underlying airways hyperresponsiveness. This suggestion is supported by the effects of K_{ATP}COs in animal models of hyperreactivity (Morley, 1994); the potency of cromakalim in human hyperresponsive airways smooth muscle is, as yet, unknown.

Interestingly, when the dose of cromakalim was increased to 1.5 mg (single dose), no significant reduction in the morning dip in lung function was observed (Williams *et al.*, 1990). The failure of the latter dose of cromakalim to improve lung function was attributed to 10 out of the 23 subjects being unable to exert maximal expiratory effort during measurements of FEV^1 values (forced expiratory volume in 1 sec) as a consequence of headache. BRL 38227, which replaced cromakalim in clinical trials, failed to elicit significant bronchodilation or reduce bronchial hyperresponsiveness to histamine or methacholine when administered as a single oral dose to asthmatics (Kidney et al., 1993); thus, not meeting the criteria set for its development in asthma. As with cromakalim, the dose-limiting side effect of oral BRL 38227 is headache, probably resulting from cerebral vasodilation (Arch *et al.*, 1992). Bimakalim, an analog of cromakalim, also lacked bronchodilatory effects following inhaled administration to mild to moderate bronchial asthmatic adult patients (Faurschou *et al.*, 1994). Whether this was a true lack of bronchial efficacy or that the dose of drug, to avoid other effects (no headaches or cardiovascular effects reported), was too low requires further investigation. Therefore, to be useful oral bronchodilators, and have the potential to reduce bronchial hyperresponsiveness, K_{ATP}COs with selectivity for airway relative to vascular smooth muscle greater than that of cromakalim or BRL 38227 are required.

BK_{Ca} have been demonstrated in high density in canine, bovine, and human airway smooth muscle (McCann and Welsh, 1986; Green *et al.*, 1991; Miura *et al.*, 1991). This has led to the proposal that openers of BK_{Ca} channels could demonstrate therapeutic benefit in regulation of tone of the respiratory system. The findings involving BK_{Ca} channels in the effects of β-agonists on airways smooth muscle (Kume *et al.*, 1989; Jones *et al.*, 1990) support this hypothesis. In human bronchi, however, NS 1619 (a BK_{Ca} channel opener; Olesen *et al.*, 1994a,b) evoked a weak relaxant effect that did not involve BK_{Ca} channels, which could call into question the utility of such drugs as bronchodilators (Templeton *et al.*, 1995). The lack of selectivity of K_{Ca}COs for the BK_{Ca} channel (see Section 2.2) has delayed progress in this area and, thus, appreciation of the true utility of such agents.

4.3. *Reproductive System*

By virtue of the smooth muscle relaxing effects, K_{ATP} channel openers may be useful in the treatment of premature labour and dysmenorrhoea (Piper *et al.*, 1990). Several K_{ATP}COs are capable of producing glibenclamide-sensitive relaxation of uterine smooth muscle of rat, both *in vitro* and *in vivo* (Piper *et al.*, 1992). Cromakalim inhibited the spontaneous phasic activity and spasmogen-induced contractions of isolated uterus from the term-pregnant rat (Hollingsworth *et al.*, 1987). BRL 38227 and pinacidil inhibited spontaneous and oxytocin-induced contractions in human isolated pregnant myometrium, obtained before and after the onset of labour (Cheuk *et al.*, 1993; Morrison *et al.*, 1993). The K_{ATP}COs were more potent in nonpregnant than pregnant human myometrium (Cheuk *et al.*, 1993). The relaxant effects of the two K_{ATP}COs in human pregnant myometrium was sensitive to glibenclamide. Thus, K_{ATP}COs may have potential as a new generation of tocolytic agents. Preferential higher potency would suggest that K_{ATP}COs would be more effective tocolytic agents in nonpregnant than pregnant women (Cheuk *et al.*, 1993). Not all women with preterm uterine contraction, however, are candidates for tocolysis (Monga and Creasy, 1995).

Although channels permeable to Rb^+ and K^+ exist in the uterus, cromakalim does not stimulate efflux of these ions in rat uterus, even though relaxant responses were sensitive to glibenclamide (Hollingsworth *et al.*, 1987). This finding was further supported in human isolated myometrium, where Rb^+ exhibited differentiation on the effects of BRL 38227 and P1060 (a pinacidil analog) on amplitude and frequency of spontaneous contraction (Criddle and Soares de Moura, 1995). In these studies, Rb^+-sensitive and Rb^+-insensitive mechanisms were identified, of which the former appeared more important in effects of P1060 than BRL 38227. Although K_{ATP}COs may demonstrate benefit in uterine-associated disorders, the involvement of K^+ channels still requires confirmation.

β-Adrenoceptor agonists, relaxin, and other uterine relaxants that increase intracellular cAMP levels, activate K_{Ca} channels in myometrial cells (Trieschmann *et al.*, 1991; Meera *et al.*, 1994; Sanborn, 1995). Iberiotoxin, a K_{Ca} channel blocker, depolarizes myometrial cells and increases phasic

contractions in rat and human myometrial preparations (Anwer *et al.*, 1993). Therefore, selective direct openers of K_{Ca} channel (iberiotoxin-sensitive) may also have therapeutic benefit in uterine-associated disorders.

Interestingly, DHS-1, derived from the medicinal herb *Desmodium adscendens* and used therapeutically in the treatment of dysmenorrhea (Ampofo, 1977), opens charybdotoxin-sensitive K_{Ca} channels (McManus *et al.*, 1993).

During labour, the forceful contractions of the uterus can occlude its blood supply, which may lead to hypoxia. Hypoxia can reduce and even abolish uterine force in both isolated rat and human uterus (Heaton *et al.*, 1993). Thus, hypoxia may contribute to uterine dystocia (inadequate uterine contraction during labour), the cause of which remains largely unknown and which often results in emergency Caesarean delivery. In a model of hypoxia involving cyanide administration to inhibit uterine force, glibenclamide reduces the K^+ efflux produced by cyanide (Heaton *et al.*, 1993), thus implicating K_{ATP} channels in this response.

More work is needed to increase our understanding of the K^+ channels in the myometrium; how they may differ from those in other tissue types and how they may be involved in different pathologic states.

In the treatment of impotence, vasodilators, injected locally, are commonly used. The established vascular smooth muscle relaxant properties of K_{ATP}COs (see Section 4.1.2) suggest that these drugs may offer an alternative to current treatment. Cromakalim and pinacidil increased $^{86}Rb^+$ efflux and inhibited spontaneous contractile activity, electrically and noradrenaline-induced contractions in rabbit isolated cavernosal tissue (Holmquist *et al.*, 1990a). Similar results were obtained for pinacidil in human isolated cavernosum (Holmquist *et al.*, 1990b). In addition, pinacidil increased whole cell K^+ current in human cultured corporal (corpus cavernosum) SMC (Christ *et al.*, 1993). Recently, cromakalim was reported to increase intracavernous pressure in a Simian monkey model, resulting in an erectile response of the penis (Trigo-Rocha *et al.*, 1994). Minoxidil was more effective in facilitating erection and produced fewer side effects than nitroglycerin, when used to treat organic impotence in men (Cavallini, 1991).

4.4. *Urinary Bladder*

Bladder hyperactivity, secondary to bladder hypertrophy or partial outflow obstruction resulting in urinary incontinence, is common, and the existing therapeutic regimens are often ineffective or poorly tolerated (Wein, 1991). Cromakalim and pinacidil relax urinary bladder smooth muscle, indicating potential in the treatment of urinary incontinence (Andersson *et al.*, 1988; Malmgren *et al.*, 1989). In isolated detrusor muscle preparations from human unstable bladder (due to urinary outflow obstruction), cromakalim inhibited elevated basal tone and spontaneous contractile activity (Foster *et al.*, 1989). Similar effects were also observed with pinacidil (Fovaeus *et al.*, 1989). K_{ATP}COs demonstrated inhibitory effects, in both human and animal tissues, on myogenic activity and contractions to a variety of spasmogens (Longman and Hamilton, 1992). The ability of K_{ATP}COs to inhibit electrically induced contractions of urinary bladder tissues have been variable and may be related to the degree of depolarization produced by neuronal stimulation in different models.

In bladder tissues from a variety of species, K_{ATP}COs (e.g., BRL 38227, pinacidil and RP 49356) increase $^{86/42}K^+$ efflux. However, as found in other smooth muscle systems, the concentrations of drug required are higher than those inhibiting myogenic activity (Longman and Hamilton, 1992). The relaxant activity and enhancement of $^{86/42}K^+$ efflux due to K_{ATP}COs are sensitive to glibenclamide.

Evaluation of K_{ATP}COs on *in vivo* urinary bladder models have been limited due to the vascular effects of the drugs. A comparison of the effects of BRL 38227, pinacidil, Ro 31-6930, RP 49356, and S 0121 did not reveal selectivity for the rat detrusor muscle over portal vein (Edwards *et al.*, 1991). A series of novel K_{ATP}COs (for example, compound ZD6169) recently have been proposed that act selectively on the urinary bladder smooth muscle, without producing significant cardiovascular effects following oral administration to rats (Grant *et al.*, 1994).

The observations in animal models have yet to be supported by clinical trials, as results of initial studies in humans were disappointing. Data from a study with pinacidil in patients with bladder hyperactivity and bladder outflow obstruction (secondary to prostatic hyperplasia) failed to demonstrate any clear improvement in bladder function (Hedlund *et al.*, 1991). Levcromakalim (BRL 38227) increased the duration of bladder contraction, but was without effect on other urodynamic parameters in patients with high spinal cord lesions (Komersova *et al.*, 1995). Hypotensive responses during this study led the authors to suggest that higher doses of the drug could only be evaluated if administered intravesically.

The relaxant effects of cromakalim and S 0121 (benzopyran K_{ATP}CO) in isolated ureter from rabbit and humans (Klaus *et al.*, 1990) suggest a benefit for K_{ATP}COs in the treatment of kidney stones by aiding their passage along the ureter (Englert *et al.*, 1988). In studies on the guinea-pig ureter, CGRP (see Section 2.3) appears to be an endogenous KCO (Santicioli and Maggi, 1994). The role of CGRP and the involvement of K^+ channels on human ureter still requires investigation.

4.5. *Gastrointestinal Tract*

Spontaneous slow-wave contractile activity and/or contractile responses to spasmogens in a variety of gastrointestinal tissues (e.g., taenia caeci, ileum, colon, muscle myenteric plexus, oesophageal, stomach) have been shown to be inhibited by K_{ATP}COs (Longman and Hamilton, 1992). These effects were associated with K^+ efflux and hyperpolarisation that involved a glibenclamide-sensitive mechanism. The animal data suggest that K_{ATP}COs may have utility in conditions associated with disturbances in gastrointestinal motil-

ity, such as irritable bowel syndrome, especially because kinetically slow K^+ channels, carrying outward current, may be responsible for gastrointestinal slow-wave activity (Benham and Bolton, 1983). Interestingly, the evaluation of such drugs in other clinical conditions has not revealed an incidence of adverse side effects on the gastrointestinal tract such as constipation; however, this would be influenced by the site of absorption of these agents. To gain a full appreciation of potential therapeutic benefit, KCOs are required that are not removed from the gastrointestinal tract.

Diazoxide, like morphine, has a protective effect on ethanol-induced gastric lesions; an effect proposed by Bhounsule *et al.* (1992) to involve K_{ATP} channels. As described in Section 4.6.2, opioid receptors are associated with K^+ channels, and glibenclamide is reported to antagonise morphine analgesia (Ocana *et al.*, 1990). In the model of gastric lesions, results obtained with glibenclamide were complicated by its action on prostaglandin production in this tissue (Bhounsule *et al.*, 1992).

Neurotensin inhibits contractions in rat and canine ileal smooth muscle by opening apamin-sensitive K_{Ca} channels (Alliexcher *et al.*, 1992; Christinck *et al.*, 1992). Therefore, SK_{Ca}COs, like K_{ATP}COs, may have utility in conditions associated with disturbances in gastrointestinal motility.

4.6. *Nervous System*

4.6.1. Peripheral. The K_{ATP}COs interfere with neurotransmission in peripheral parasympathetic neurones in the airways and the gastrointestinal tract (Longman and Hamilton, 1992; Section 4.2). This has led to suggestions of a presynaptic site of action, whereby the K_{ATP}COs control the release of neurotransmitter. In contrast, cromakalim, nicorandil, and pinacidil failed to exert an inhibitory effect on noradrenaline release in rat isolated mesenteric artery (Fabiani and Story, 1994). The presynaptic inhibitory role of K_{ATP}COs, therefore, appears to be selective for parasympathetic (cholinergic) innervation.

Interestingly, cromakalim and pinacidil inhibited nicotinic acetylcholine receptor-mediated and voltage-dependent catecholamine secretion from bovine adrenal chromaffin cells (Masuda *et al.*, 1994). Thus, K_{ATP}CO-sensitive K^+ channels could be involved in regulation of catecholamine secretion mainly indirectly through VOCs.

4.6.2. Central. Potassium channels play a pivotal role in the control of neuronal excitability, action potential, and neurotransmitter release within the CNS (Hille, 1984; Cook, 1990). Activation of a variety of receptors (e.g., opioid, 5-HT_1, somatostatin, α_2-adrenoceptors) by the appropriate neurotransmitter alters the flux of K^+ ions from neurones (North, 1989). Because of this role in normal CNS physiology, derangements in the function of K^+ channels may underlie several CNS diseases. Studies of the distribution of binding sites for the three ligands, [^{125}I]-iodoglyburide, [^{125}I]-apamin and [^{125}I]-charybdotoxin, as markers for K_{ATP}, SK_{Ca} and BK_{Ca} (or voltage-gated K^+) channels, respectively, was dramatically different in the rat brain (Gehlert and Gackenheimer, 1993). These data indicate that pharmacological modulation of these K^+ channel subtypes should result in distinctly different effects on brain function. [^{125}I]-Iodoglyburide binding exhibits a very broad distribution in brain, being found in a majority of brain regions. The globus pallidus and the zona reticulata of the substantia nigra (involved in movement coordination) contain the highest density of binding. Openers of K_{ATP} channels have been suggested to exert a protective effect on ischemic tissue by reversing the ischemia-induced depolarization (see below). Therefore, K_{ATP}COs may find utility as neuroprotectant drugs, and the broad distribution of these channels would indicate that these drugs would have an effect on most neuronal populations in the brain. Although K_{ATP}CO ligands, [^{3}H]P1075 and [^{3}H]cromakalim, are available, a profile of the binding site(s) distribution within the CNS is not published as yet. [^{125}I]-Apamin binding suggested that SK_{Ca} channels are associated primarily with cell bodies and dendritic spines, rather than nonneuronal elements. Localization in the cerebral cortex and hippocampus would suggest that this channel may have a role in the processing of memory. Interestingly, a loss of [^{125}I]apamin binding sites has been reported in the subiculum and CA_1 neurones of hippocampal tissue obtained from postmortem Alzheimer's disease patients (Ikeda *et al.*, 1991). A reasonable hypothesis is that openers of the SK_{Ca} channel may confer a neuroprotective effect; however, development of appropriate chemical molecules and pharmacological tools is awaited. The highest levels of [^{125}I]-charybdotoxin binding sites were found in the white matter-containing regions, such as the lateral olfactory tract and fasciculus retroflexus. This suggests that the charybdotoxin-sensitive K^+ channel is present on axons and may modulate nerve conductance in these regions.

Studies *in vitro* and in animal models indicate the potential clinical utility of K_{ATP}COs for diseases of the CNS.

During anoxic conditions, neuronal depolarization is due, at least, to the release of large concentrations of excitatory amino acids, such as glutamate, which may be involved in long-term ischemia-induced damage to the brain (Miller, 1990). In *in vitro* experiments, diazoxide and somatostatin were shown to prevent anoxia-induced depolarization of CA3 hippocampal neurons following the opening of K^+ channels (Ben-Ari *et al.*, 1990); these effects were inhibited by pretreatment with glyburide. The authors proposed that K_{ATP}COs may prevent anoxia-induced damage to hippocampal neurons by inhibiting the release of excitatory amino acids. This suggestion is supported by the finding that levcromakalim and RP 52891 blocked ischemia-induced glutamate release in rat hippocampal slices (Zini *et al.*, 1993). In addition, the K_{ATP}COs levcromakalim, nicorandil, and pinacidil blocked ischemia-induced expression of the genes *c-fos* and *c-jun* and of the mRNAs for 70-kDa heat-shock protein and the form of the amyloid β-protein precursor, including the Kunitz-type protease inhibitor domain in rat hippocampus (Heurteaux *et al.*, 1993).

The movement disorders associated with Parkinson's dis-

ease are due to a selective loss of dopaminergic neurons in the substantia nigra. The highest density of $K_{ATP}s$, as judged by autoradiographic studies with [^{125}I]-iodoglyburide, in the brain is found in the substantia nigra (Gehlert and Gackenheimer, 1993). Sulphonylurea binding studies, however, provide essentially only indirect evidence of $K_{ATP}s$. Sulphonylureas or increasing extracellular glucose increase the release of [^{3}H]-GABA from the substantia nigra, effects that are inhibited by K_{ATP}COs (Schmid-Antomarchi *et al.*, 1990). GABAergic pathways to other CNS structures (e.g., raphe nuclei) are also modified by K_{ATP}COs. Schmid-Antomarchi *et al.* (1990) noted that the order of potency of the K_{ATP}COs (levcromakalim > nicorandil > cromakalim > diazoxide > pinacidil) was found to be different from that in either the pancreatic β-cell or in smooth muscle, possibly indicating a difference between the target K^+ channel in this brain region and that in other tissues. The K_{ATP} channel in neuronal tissue is not the classical (Type 1) channel found in pancreatic β-cells or heart, but a large-conductance nonrectifying version (Type 2; Ashcroft and Ashcroft, 1990).

The genesis and propagation of nonphysiologic electrical impulses are the hallmark of epilepsy. Thus, the hyperpolarization (and restraining) of excitable cells through the opening of K^+ channels could demonstrate therapeutic benefit in this setting. The K_{ATP}COs cromakalim and RP 52891 (aprikalim) reduced seizures in genetically epileptic rats (Gandolfo *et al.*, 1989) and pentylenetetrazole-induced seizures in mice are blocked by intracerebroventricular administration of cromakalim, but not pinacidil (Del Pozo *et al.*, 1990). In a diltiazem-induced model of tonic-clonic seizures in the rat, cromakalim completely inhibited both EEG and behavioural seizures, and pentobarbitone only prevented behavioural activity (Popoli *et al.*, 1991). K_{ATP}COs counteract anoxic hyperexcitability, but not 4-aminopyridine-induced epileptiform activity in the rat hippocampal slice (Mattia *et al.*, 1994), suggesting such drugs might be useful in the treatment of seizures occurring in the setting of status epilepticus or cerebrovascular disease. High concentrations of cromakalim inhibit electrically induced epileptiform discharges in guinea-pig hippocampus (Alzheimer and ten Bruggencate, 1988). Finally, the antiepileptic drug carbamazepine increases potassium currents in rat cortical neurons (Zona *et al.*, 1990). However, whether or not this property is linked to the pharmacological actions of the drug requires further investigation. A similar property (K^+ channel opening), which may be of clinical importance, has been reported to be exhibited by oxcarbazepine (McLean *et al.*, 1994).

Opioids exert their analgesic effects by binding to opiate receptors, which leads to opening of K^+ channels and neuronal hyperpolarization (North, 1989). Morphine-induced antinociception in mice tail-flick tests is mediated by the opening of K_{ATP} channels (Ocana *et al.*, 1993). These observations would suggest a potential role for KCOs as analgesics. Intrathecal administration of the K_{ATP}COs BRL-38227, minoxidil, and diazoxide produced antinociception in the tail-flick test in mice (Welch and Dunlow, 1993). The K_{ATP}COs were not cross-tolerant to the effects of morphine in this model. This led to the suggestion that the K_{ATP}COs and opioids probably do not act on a common site, but could have a common second messenger (Welch and Dunlow, 1993). A dose-dependent increase in the effects of morphine on the hot-plate and tail-flick tests were obtained following i.c.v. administration of pinacidil to rats (Vergoni *et al.*, 1992).

Benzopyran derivatives of cromakalim (e.g., SR 46142A) have been claimed to exert antidepressant activity in animal tests (improve swimming performance in mice) in the absence of a cardiovascular effect (Garcia *et al.*, 1990). Similar results were observed with a series of amidochromanols (Fourcelet, 1990). This would suggest that modifications of the benzopyran nucleus of cromakalim allows tissue selectivity (CNS over vascular smooth muscle) to be achieved. Whether or not the antidepressant effects of SR 46142A (and related compounds) are mediated by modulation of K^+ channels remains to be confirmed.

These studies are encouraging, but neuronal tissue-selective agents that cross the blood–brain barrier are needed to realize the potential clinical applications of K_{ATP}COs in CNS disorders. In addition, the promiscuity of K_{ATP}COs invites adverse CNS effects; for example, cromakalim, like d-amphetamine, enhances spontaneous locomotor activity in the rat by a glibenclamide-sensitive mechanism (Amalric *et al.*, 1992).

BK_{Ca} channels also play a role in the function of neuronal cells (Hille, 1984; Cook, 1990). The recent identification of compounds (e.g., NS 1619, see Section 2.2) that activate BK_{Ca} channels will allow determination of their utility as potential therapeutic agents. In rat cerebellar granule cells, NS-004 activates a 187 pS Ca-dependent K^+ channel that is blocked by charybdotoxin (Olesen *et al.*, 1994a). NS 1619 activates the BK_{Ca}, but not K_{ATP} channels, in membrane patches isolated from rat ventromedial hypothalamic neurones (Sellers and Ashford, 1994). Although K_{ATP} channels are present in rat ventromedial hypothalamic neurones, BRL 38227, cromakalim, and pinacidil all failed to evoke an effect on these channels in excised membrane patches (Sellers *et al.*, 1992). The glucose-sensitive cells in the ventromedial hypothalamus are involved in the control of appetite and are often regarded as the satiety center (Morley, 1980; Blundell, 1992). Thus, activation of BK_{Ca} channels would decrease hypothalamic firing and reduce the sensation of satiety.

4.7. *Skeletal Muscle*

In skeletal muscle, K_{ATP} channel activity has been shown to increase upon intracellular acidification (Davies, 1990). Falls in intracellular pH reduce the inhibitory effect of ATP on K^+ channels in frog skeletal muscle. This could mean that during increased muscle exercise and consequent lowering of pH, K_{ATP} channel-induced hyperpolarization could compensate for a decrease in electrical excitability and prevent spontaneous contractions from occurring.

The K_{ATP}COs, cromakalim, pinacidil, and RP-49356,

increased opening time of a glibenclamide-sensitive K^+ channel in mouse skeletal muscle (Weik and Neumcke, 1990). Diazoxide activated K_{ATP} channels in smooth muscle and pancreatic cells, but had very little effect in skeletal muscle, even at very high concentrations (Weik and Neumcke, 1990). Interestingly, this sulphonamide (thus, structurally related to the suphonylureas) inhibited ATP-sensitive channels in ventricular muscle cells (Faivre and Findlay, 1989). Thus, the different effects of diazoxide suggest that the K^+ channels in mouse skeletal muscle resemble those in cardiac cells more than those in smooth muscle and pancreatic β-cells (Lawson and Hicks, 1993). Due to diverse effects of pinacidil in mouse skeletal muscle, a model of the K_{ATP} channel was proposed with a binding site for the K_{ATP}CO and two sites for nucleotides, one activatory and one inhibitory (ATP or ADP can occupy either site; Hehl and Neumcke, 1994). Pinacidil activates the channel and displaces the blocker from the inhibitory site, only if the activatory site is occupied.

Cromakalim enhanced K^+ efflux in human skeletal fibres, an effect that was blocked by tolbutamide (Spuler *et al.*, 1989), suggesting K_{ATP}COs could have some role in the treatment of pathologic muscle fatigue or paralysis resulting from excessive membrane depolarization. Interestingly, cromakalim, pinacidil, and RP-49356 evoked larger hyperpolarizations in skeletal muscle fibres from patients with myotonic dystrophy or hypokalaemic periodic paralysis than in those from normal volunteers (Spuler *et al.*, 1989; Quasthoff *et al.*, 1989; Grafe *et al.*, 1990). K_{ATP}COs, however, increased open probability of an ATP-sensitive and an ATP-insensitive K^+ channel in human skeletal muscle (Quasthoff *et al.*, 1990); the effect of the K_{ATP}COs on both channels were blocked by glibenclamide.

Ischemia-induced damage in a rat skeletal muscle, like cardiac (Section 4.1.1) and neuronal (Section 4.6.2) ischemia, has been shown to be prevented by cromakalim (Hatton *et al.*, 1991). This result, and the observation that cromakalim restores the membrane potential of depolarized human skeletal muscle fibres (Spuler *et al.*, 1989), indicate that K_{ATP}COs may be useful for the treatment of peripheral vascular disease (see Section 4.1.1). In rat skeletal muscle, Angersbach and Nicholson (1988) demonstrated that K_{ATP}COs, but not Ca^{2+} antagonists or hydralazine, selectively increase blood flow to collateral vessels in a previously ischemic limb. Woolcotch *et al.* (1993), however, suggested that K_{ATP}COs would not be beneficial in treatment of skeletal muscle ischemia in vivo, but may be useful in preserving skeletal muscle function in cases of ischemia followed by reperfusion.

Studies in human muscle have implicated SK_{Ca} (apamin-sensitive) channels in the condition myotonic muscular dystrophy, characterised by muscle stiffness (Renaud *et al.*, 1986). Although K^+ channel subtypes other than K_{ATP} channels exist in skeletal muscle (SK_{Ca}, BK_{Ca}, delayed rectifier K^+ channel; Wareham, 1992), however, selective openers are awaited to determine their role and the therapeutic potential of activation.

4.8. *Hair Growth*

The occurrence of hypertrichosis during antihypertensive treatment with minoxidil (Campese, 1981) led to the subsequent evaluation of the drug (applied topically) to enhance hair regrowth in areas of baldness (Clissold and Heel, 1987). Topically administered minoxidil enhances hair growth in certain forms of male pattern baldness. Although this effect has been suggested to involve K^+ channels, hypertrichosis occurs in 80–100% of minoxidil-treated patients, but only 2–13% of pinacidil-treated patients. There is no evidence that cromakalim, nicorandil, or RP-49356 stimulate hair growth. In SMC, minoxidil (sulphate) has been suggested to open a K^+ channel that is not recognised by other K_{ATP}COs (see Section 2.1). K_{ATP}COs stimulate DNA synthesis in mouse epidermal keratinocyte and whole hair follicle cultures (Harmon *et al.*, 1993). In cultured whisker follicles, minoxidil, but not P1075 (pinacidil analog), preserved the root sheath; however, both drugs stimulated cysteine incorporation in follicles (Waldon *et al.*, 1993). The root sheath may be the target for minoxidil; thus, stimulating hair growth through a direct effect on the hair follicle.

4.9. *Intraocular Pressure*

Repeated topical application of pinacidil, cromakalim, or nicorandil lowers intraocular pressure (IOP) of rabbits, suggesting a potential benefit of K_{ATP}COs in eye disorders such as glaucoma (Godtfredsen, 1989). In an isolated arterially perfused bovine eye preparation, pinacidil caused a sustained decrease in IOP, with no effect on arterial perfusion pressure (Millar and Wilson, 1991); thus, suggesting that relaxation of resistance vessels is not involved in the fall in IOP due to the K_{ATP}CO. Whether these effects in the eye may be attributed to enhanced K^+ ion movement and consequent relaxation of smooth muscle or are the result of K^+ channel modulation in epithelial cells requires further investigation.

5. CONCLUSIONS

Synthetic molecules that 'directly' activate K^+ channels have led to a new direction in the pharmacology of ion channels. The identification of the K^+ channel opening property of cromakalim initiated major research efforts in the search for other such agents and in the determination of the specific channel(s) involved. The existence of so many different subtypes of K^+ channels has been an impetus in the search for new molecules that would have different profiles and channel selectivities (e.g., K_{ATP}, BK_{Ca}). The availability of an increasing number of KCOs, exogenous and endogenous, should facilitate more detailed study of these channels under both normal and certain pathophysiological conditions. The decrease in the excitability of cells that follows K^+ channel opening offers a broad clinical potential for drugs with this property in a number of pathologic conditions. Consequently, therapeutic roles of KCOs can be envisaged in disorders of a wide range of cells; for example, vascular and nonvascular smooth muscle,

cardiac, neuronal, and skeletal cells. Although lack of selectivity of current compounds remains one of the major hurdles in this area, advances in prototype KCOs and our knowledge of K^+ channel pharmacology is encouraging. Thus, the development of KCOs that will provide positive results in extensive clinical trials to give an appreciation of the full therapeutic potential are eagerly awaited.

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